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POLYPEPTIDE RENIN INHIBITORS - CONTG. CAT. MODIFIED STATINE RESIDUE

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(71) Applicant: Smithkline, Michael
10 Harris Drive
Ruislip Middlesex (GB)

(71) Applicant: Jones, David Michael
88 Woodlawn Avenue
Haven Middlesex (GB)

(71) Applicant: Hallet, Allan
88 Longdown Road
Chessington Surrey (GB)

(72) Inventor: Smithkline, Michael
10 Harris Drive
Ruislip Middlesex (GB)

(72) Inventor: Jones, David Michael
88 Woodlawn Avenue
Haven Middlesex (GB)

(72) Inventor: Hallet, Allan
88 Longdown Road
Chessington Surrey (GB)

(74) Representative: Fennell, William Robert et al.
PHILLIPS & LEIGH 7 Staple Inn Holborn
London WC1V 7QF (GB)

(57) Abstract

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EP 0 104 041 A1

ENZYME INHIBITORS

The invention relates to renin-inhibiting peptide analogues.

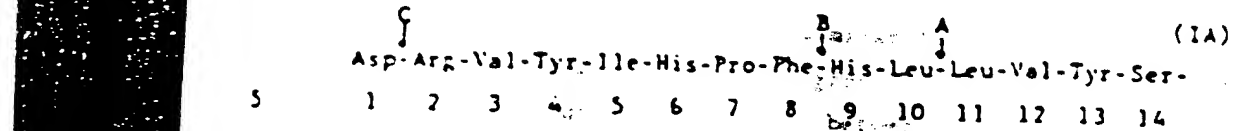
BACKGROUND

Renin is a natural enzyme, disorders in relation to which are implicated in many cases of hypertension. It is released into the blood from the kidney, and cleaves from a blood glycoprotein a decapeptide known as angiotensin-I. Circulating angiotensin-I is cleaved in lung, kidney and other tissues to an octapeptide, angiotensin-II, which raises blood pressure both directly by causing arteriolar constriction and indirectly by stimulating release of the sodium-retaining hormone aldosterone from the adrenal gland and thus causing a rise in extracellular fluid volume. The latter effect is caused by angiotensin-II itself or a heptapeptide cleavage product angiotensin-III.

Inhibitors of renin have therefore been sought, with two ends in view, first the provision of a diagnostic agent for identification of cases of hypertension due to renin excess, and secondly the provision of an agent for control of hypertension in such cases.

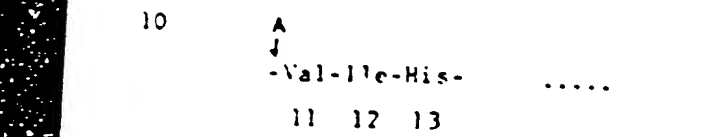
The present inventors' approach has been to consider the peptide sequence characterising the natural renin substrate at its binding site, and to seek peptide analogues sufficiently similar to bind to the enzyme, in competition with the natural substrate, but sufficiently dissimilar to it to be cleaved slowly or not at all. Such analogues will block the action of the enzyme and attack the hypertension at source.

Renin is specific to a particular bond in the substrate, the N-terminal sequence of which in the horse is for example:



(IA)

as found by L.T. Skeggs et al J. Exper. Med. 106 439 (1957). Human renin substrate has a different sequence recently discovered by D.A. Tewkesbury et al Biochem. Biophys. Res. Comm. 99 1311 (1981)

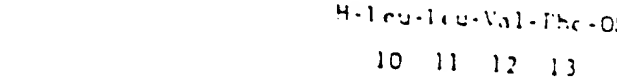


(IB)

the sequence to the left of the arrow A being as in formula (IA).

Cleavage at A gives angiotensin-I; subsequent cleavage at the Phe-His bond at B gives angiotensin-II; and cleavage subsequently again at the Asp-Arg bond at C gives angiotensin-III.

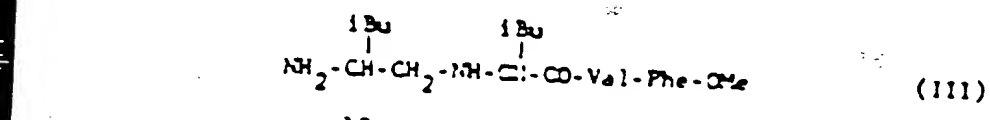
Peptides similar to certain partial sequences of the substrate have been shown to act as inhibitors of renin in vitro. An example is the tetrapeptide ester (the relation to the substrate residues being indicated by numbering):



(II)

proposed by Kokubu, Nature, 217 456 (1968) but it is inactive in vivo, because of binding to plasma proteins and rapid attack by natural peptidases.

One of the present inventors undertook some years ago a development of Kokubu's work, seeking a renin inhibitor active in vivo, in which analogues of peptides similar to Kokubu's were made but having a methylene imino group $-CH_2-NH-$ in place of the peptide link $-CO-NH-$ between the leucine residues. One of these analogues was:



(III)

which is the tetrapeptide (I) modified at the Leu-Leu link, leucine of course being

(IV)

This analogue (III) was the first effective in-vivo inhibitor of renin and was shown to have significant antihypertensive action in Goldblatt hypertensive rats (Parry, Russell and Szelke p. 541 in "Chemistry and Biology of Peptides" Ed. Meienhofer, Ann Arbor Science Publishers 1972). Little or no attention has however been paid to the work, which the authors themselves were unable to pursue, in spite of considerable activity in the general field of substrate-based inhibitors for renin, reviewed for example by Haber & Burton, Federation Proc. 38 No. 13 2768-2773 (1979).

THE INVENTION

The present invention is a development of the above work, to which the inventors were stimulated by consideration of the acid protease inhibitor pepstatin further considered later herein. It contains the acid statine $NH_2CH(iBu)CH(OH)CH_2COOH$, an amino acid (though not an alpha amino acid as the common natural amino acids are) and appeared to the inventors to offer scope for work related to though on different lines from their work on peptide analogues disclosed for example in their U.S. patent application Serial No. 290 620 filed 5 August 1981 (published as European Patent Specification No. 0 045 665 on 10th February 1982).

In those analogues a peptide bond is represented by other links corresponding to partial or complete reduction at the carbonyl group and/or replacement of the nitrogen (-NH-) group by a methylene group. The inventors have however now surprisingly found that notional replacement of the carbonyl group by a number of 2-, 3- or 4-carbon groups including but by no means restricted to the group seen in statine gives renin-inhibiting peptide analogues of high specificity and activity. This is so even though the structural relation to the polypeptide that is the natural substrate of renin is less than previously. Low activity would be expected and, further, loss of specificity as for example with pepstatin itself.

Thus behind both inventions is a concept of modified peptide structures related to the peptide sequence at the site of action of renin on the natural substrate, by substitution at the site of cleavage, but the two approaches are distinct.

Optionally further in the present invention there is isosteric substitution, or other modification, at other positions to increase stability or to modify the properties of the final peptide, for example its solubility under physiological conditions or its resistance to degredation in vivo. Such modification may for example be by incorporation of residues other than those of the natural L-amino acids; by protection of the N-terminus with acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or other groups; or by conversion of the C-terminal carboxyl to another functional group, e.g. the corresponding alcohol, present as such or in ether or ester form.

THE PRESENT COMPOUNDS DEFINED

General reference to amino acids and amino acyl residues and side chains in both the description and claims herein is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino.

The compounds of the present invention, showing desirable renin inhibitory action, are of the general formula:

$$\underset{6\ 7\ 8\ 9\ 10,11\ 12\ 13}{X\text{-}Y\text{-}Pro\text{-}Phe\text{-}His\text{—}A\text{—}B\text{—}Z\text{—}W} \qquad (V)$$

or the partial sequences:

X-A-B-Z-W (V) (i)

X-His-A-B-Z-W (V) (ii)

X-Phe-His-A-B-Z-W (V) (iii)

X-Pro-Phe-His-A-B-Z-W (V) (iv)

where:

Pro, Phe, and His may be in substituted form, e.g. carrying OH, F, Cl, Br or Me;

X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, benzyloxycarbonyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1-C_5); or an D- or L- amino acyl residue (especially Pro), which may itself be N-protected similarly:

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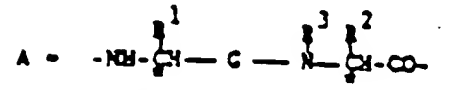
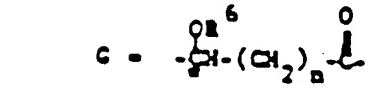
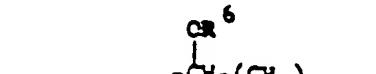
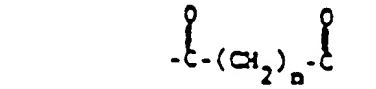
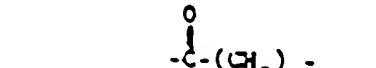
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;

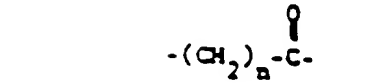
A =  (VI) where

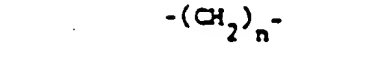
C =  (VII) with n = 0, 1, 2, 3

 (VIII) n = 1, 2, 3, 4

 (IX) n = 0, 1, 2, 3

 (X) n = 1, 2, 3, 4

 (XI) n = 1, 2, 3

 (XII) n = 3, 4

and where the configuration at asymmetric centres * is either R or S, R^1 and R^2, the same or different, being = ^{i}Pr (isopropyl), ^{i}Bu (isobutyl), Bzl (benzyl) or other amino-acid side chain preferably lipophilic or aromatic;

R^3 = H; lower alkyl (C_1-C_5); or t-butyloxycarbonyl, benzyloxycarbonyl, ring substituted benzyloxycarbonyl, $-SO_2PH$, $-SO_2C_6H_4CH_3(p)$, formyl or other N-protecting group including lower acyl (C_1-C_5) generally;

R^6 = H, or lower alkyl, lower acyl, benzyl, tetrahydropyranyl, or other hydroxyl protecting group;

B = D- or L- Val Leu or Ile or other D- or L- lipophilic amino-acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D- aromatic amino-acyl residue;

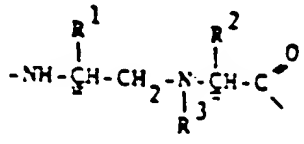
and W = i)-OH as such or in protected ester form e.g. as $-OR^4$ where R^4 = lower alkyl primarily C_1-C_5 and particularly ^tBu, or cycloalkyl primarily C_3-C_7, or Bzl, or other ester forming group; or ..., $-NH_2$, as such or in protected amide form as $-NHR^5$ or $-N(R^5)_2$ (where R^5 = an N protecting or other substituent group e.g. lower alkyl as for R^4 and $(R^5)_2$ = two such groups or e.g. cyclo-alkyl, primarily C_3-C_7), or as $-NH-(CH_2)_n-Q$ or $-NR^5-(CH_2)_n-Q$ (where n = 2 to 6 and Q = NH_2 or $-NH-C(=NH)-NH_2$ and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R^5)_2$); or iii) a D- or L- (D- or L-) serine or lysine, arginine or other basic amino-acyl residue as such or in amide form, substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case

may be; or iv) an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above

or

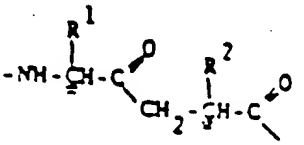
Z + W = an alcohol derived from L- or D-Tyr, Phe or His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds, for example by reduced $-CH_2-NH-$, keto, $-C(=O)-CH_2-$, hydroxy, $-CH(OH)-CH_2-$, or hydrocarbon $-CH_2-CH_2-$ isosteric links in the form:



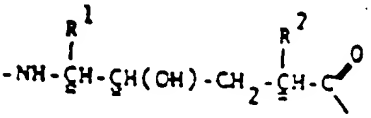
(XIII) "reduced" isostere bond

or



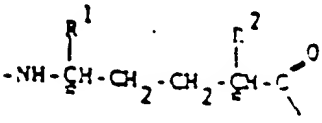
(XIV) "keto" isostere bond

or



(XV) "hydroxy" isostere bond

or



(XVI) "hydrocarbon" isostere bond

where the significance of *, R^1, R^2 and R^3 is as before;

and said polypeptide further being in free form or in protected form at one or more remaining amino or amide (including peptide) nitrogen, carboxyl, hydroxy or other reactive groups, or in salt form at amino, imidazole or carboxyl groups, in particular as their physiologically acceptable acid addition salts at basic centres.

The above compounds may in particular be those related to the substrate sequence in the horse (B = Val at position 12) or those related to the substrate sequence in man (B = Ile at position 12). Particular groups of these compounds are set out in the formulae below, either:

(A) X-Y-Pro-Phe-His—A—Val-Z-W (VA)

6 7 8 9 10,11 12 13

where

X, Y, Pro, Phe and His are as before

A is as before except that

R^1 and R^2, the same or different = ^iBu (isobutyl), ^iPr or Bzl (benzyl) or other amino-acid side chain preferably lipophilic or aromatic

R^3 = -H; or -CH_2Ph, -$SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group

R^4 = lower alkyl, lower acyl, benzyl, tetrahydropyranyl or other O-acyl protecting group

Z = Ile, Leu or other basic or aromatic amino-acyl residue;

W = -OH or such or in protected ester form as -OR^6 where

R^6 = lower alkyl (preferably C_1-C_5 and particularly ^tBu), or Bzl, or other ester forming group; or -NH_2 or such or in protected amide form as -NHR^5 or -$N(R^5)_2$ (R^5 = an N-protecting group e.g. lower alkyl as for R^6; or -NH- such or e.g. cyclo-alkyl, preferably C_{5-7}

an L- or D- amino-acyl residue e.g. a serine or basic amino-acyl residue as such or in amide form or in protected amide or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case may be; or an amino acid alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above

or

Z + W = an alcohol derived from Tyr or Phe or other L- or D- aromatic amino acyl residue as such or protected in ester or ether form as above; or:

(B) X-Y-Pro-Phe-His—A—Ile—Z—W (VB)

6 7 8 9 10,11 12 13

where

X, Y, Pro, Phe and His are as before

A is as before except that

R^1 = ^{i}Bu (isobutyl) or Bzl (benzyl) or other amino-acid side chain preferably lipophilic or aromatic

R^2 = ^{i}Pr (isopropyl), and

R^3 = -H; or $-SO_2Ph$, $-SO_2C_6H_4CH_3(p)$, Boc, formyl or other N-protecting group

R^6 = lower alkyl, lower acyl, benzyl, tetrahydropyranyl, or other hydroxyl protecting group

Z is as before

W is as in formula (VA)

or Z + W = an alcohol derived from the aromatic residues specified for Z before, as such or protected in ester or ether form as specified therein.

The numbering of residues in formulae (V), (VA) and (VB) shows the correspondence with the renin substrates themselves, but without limitation of the generality of the formulae.

Substitutes for Pro Phe and His above may for example be: (1) for Pro 4-hydroxyproline (HPro) or pGlu (2) for Phe: Tyr, Phe(4-Cl), Phe(4-F) (3) for His: His(Me), Spinacin.

Reference to basic and aromatic amino acids above, and to amino acids with lipophilic side chains includes but is not restricted to the common amino acids of those classes, viz:

Basic:	Arginine Lysine Histidine		
Aromatic:	Phenylalanine Tyrosine Tryptophan Histidine		
Lipophilic:	Leucine Isoleucine Valine	rnenylalanine Cyclohexylalanine) Adamantylalanine)	unnatural

Suitable amino acyl residues X may for example be those of: D or L Pro, Val or Ile; Gly.

Where a peptide bond in addition to that corresponding to the Leu-Leu or Leu-Val bond in the natural renin substrate is modified, the 7,8 and 8,9 positions i.e. the Pro-Phe and Phe-His bonds in formula V are preferred, or possibly both of these positions, and it is further preferred that the substitution should be

(VII)

i.e. as a "reduced" isostere bond XIII, where R^3 is as set out above. The alternative isosteric substitutions set out herein may however be used.

Protective or substituent groupings as mentioned above may be any of these known in the polypeptide art, amply disclosed in the literature and not requiring discussion at length here. Generally the selection of the groups is according to their function, some being primarily intended to protect against undesired reaction during synthetic procedures while the N- and C- terminal substituents are for example directed against the attack of enzymes on the final compounds or to increase their solubility and hence physiological acceptability. All these functions are generally within the terms "protective group" or the like used herein, including the claims. It is in particular possible for one or more remaining peptide bonds in the compounds of formula (V), (VA) or (VB) to be N-substituted.

STATINE

A particular representative of the group

(XVIII)

above is the S,S-statine residue (Sta)

(XIX)

Statine itself is a known compound occurring in nature in the protease inhibitor pepstatin isolated in 1970 by Japanese workers from various Actinomycetes (Morishima et al J. Antibiot. 13 No. 5 259-265 (1970)) of formula:

iso-valeryl-L-valyl-L-valyl-S,S-statyl-L-alanyl-S,S-statine (XX)

or, in the usual notation:

iso-valeryl-Val-Val-Sta-Ala-Sta-OH (XXI)

It inhibits acid proteases in general, so called because the catalytic functional groups are carboxyl groups of aspartic acid residues (as opposed to serine residues in serine proteases). Pepsin, cathepsin D, chymosin and renin are some of the representatives of this class of enzymes, and all are inhibited by pepstatin.

Incorporated however for example in the compound

H-His-Pro-Phe-His-Sta-Val-Ile-His-OH (H 176) (XXII)

statine when tested by the methods in our published European specification gives IC_{50} = 0.016 μM against human renin. This compound is the subject of Example 1 below.

APPLICATIONS

The invention further lies

i) In a diagnostic test for high renin states, blood pressure falling most when renin is high, and a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), by the administration of a polypeptide analogue as above followed by monitoring of blood pressure, and such polypeptide analogues when for such use, and

ii)In the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, by the administration of a renin-inhibiting amount of a polypeptide analogue as above, and such polypeptide analogues when for such use.

The long and short term response of blood pressure to renin inhibitors is predictive of surgical outcome. In all cases single and repeated doses and any conventional form of pharmaceutical composition may be used, for administration by intranasal or oral route, injection, or any other means as convenient. Amounts may for example be 0.001 to 10 mg/kg body weight daily more usually 0.01 to 1mg, according to the potency of the analogue and the severity of the condition. Dosage unit compositions may contain such amounts or submultiples thereof to make up the daily dose.

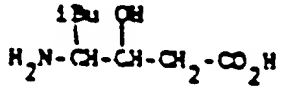
(Dosages herein are related to the free base content where compounds are in salt form.)

SYNTHETIC METHODS

The inventors have developed synthetic methods for the isosteric replacement of the peptide bond -CONH- with alternative groups, specifically $-CH_2-NH-$ (reduced), $-CH_2CH_2$ (hydrocarbon), $-C(=O)-CH_2-$ (keto) and $-CH(OH)-CH_2-$ (hydroxy) isosteres as referred to earlier herein, see e.g. Szelke, et al, pp. 57-70 in "Molecular Endocrinology" Vol. 1, Editors: MacIntyre and Szelke, Elsevier, Amsterdam 1977; Hudson, Sharpe and Szelke, U.S. Patent 4 198 398 "Enkephalin Analogues"; and Szelke, Jones and Hallett (Ferring Pharmaceuticals Ltd. and Ferring AB) in the published European specification already referred to.

Reference may be made to these publications for general discussion of such isosteric replacement and, in the European specification, for discussion in relation to renin inhibitors particularly. Reaction sequences for the preparation of peptide analogues applicable in the context of the present invention, apart from the link at A, are given there.

Turning therefore to the link at A, first there are several known syntheses of statine (XXIII below) available, e.g. that by D.H. Rich et al, J. Org. Chem. 1978, 43, p.3624 and references quoted therein. Deoxystatine (XXIV below) has also been described (D.H. Rich et al, BBRC 1977, 74, p.762), as has the synthesis of nor-statine (XXV below) and its various analogues (R. Nishizawa et al, J. Med. Chem. 1977, 20, p.510).



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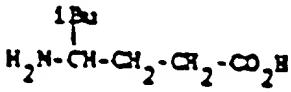

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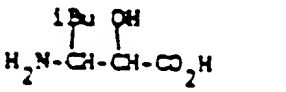
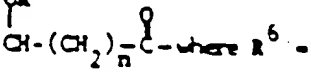
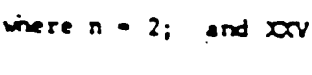
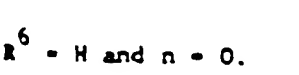


(XXV)

Of these, comparing with the possibilities for C given earlier, XXIII has C =  where R^6 = H and n = 1; XXIV has C =  where n = 2; and XXV has C =  where R^6 = H and n = 0.

For example, the following specific compounds have been made:

H-His-Pro-Phe-His-Sta-Val-Ile-His-OH (H-176) (XXII)

H-Pro-His-Pro-Phe-His-Sta-Val-Ile-His-Lys-OH (H-189)(XXXVI)

Their preparation was carried out by the procedures generally as described in our published European specification, and given in detail in the Examples which appear below. Statine was incorporated in the form of N^{im}-Boc-statine, using DCCI and HOBt as coupling reagents.

Other structures for G of particular value are:

	1	$-\overset{*}{\underset{}{C}}H(OH)-CH_2-CO-$	(XXVI)
	2	$-\overset{*}{\underset{}{C}}H(OH)-CH_2-CH_2-$	(XXVII)
	3	$-C(=O)-CH_2-CO-$	(XXVIII)
5	4	$-CH_2-CH_2-CO-$	(XXIX)
	5	$-\overset{*}{\underset{}{C}}H(OH)-CO-$	(XXX)
	6	$-\overset{*}{\underset{}{C}}H(OH)-CH_2-$	(XXXI)
	7	$-C(=O)-CO-$	(XXXII)
	8	$-CH_2-CO-$	(XXXIII)
10	9	$-C(=O)-CH_2-$	(XXXIV)

Syntheses of these, generally applicable but given by way of example in the context of the detailed synthesis of Example 1 and thus giving octapeptide analogues of H-176 containing the various structures, are respectively as follows, referring to the reaction schemes given below:

1 By coupling of Boc-Sta-OH to the appropriate protected peptide, which in the synthesis for example of H-176 is H-Val-Ile-His(Bom)-O(R)(LVI) ((R)= resin support)

2 By coupling compound L (Scheme II below, R = H) to H-Ile-His-(Bom)-O(R) (LVII)

3 By oxidising Boc-statine with pyridinium dichromate and coupling the resultant keto acid

$Boc\text{-}NH\text{-}CHR^1\text{-}CO\text{-}CH_2\text{-}CO_2H$ (XXXVII) (R^1 = ^iBu)

to the tripeptide ester LVI above

4 By coupling Boc-deoxy-statine

$Boc\text{-}NH\text{-}CHR^1\text{-}CH_2\text{-}CH_2\text{-}CO_2H$ (XXXVIII) (R^1 = ^iBu)

prepared according to Rich et al, BBRC 1977, 74, p. 762, to the tripeptide ester LVI.

5 By coupling Boc-nor-statine (obtained according to R. Nishizawa et al, J. Med. Chem. 1977, 20, p. 510) to the tripeptide ester LVI.

6 By coupling compound XLVI (Scheme I below, R = H) to the dipeptide ester LVII above.

7 By oxidising Boc-nor-statine to the corresponding keto acid

$$Boc\text{-}NH\text{-}CHR^1\text{-}CO\text{-}CO_2\text{-}H \qquad (XXXIX)$$

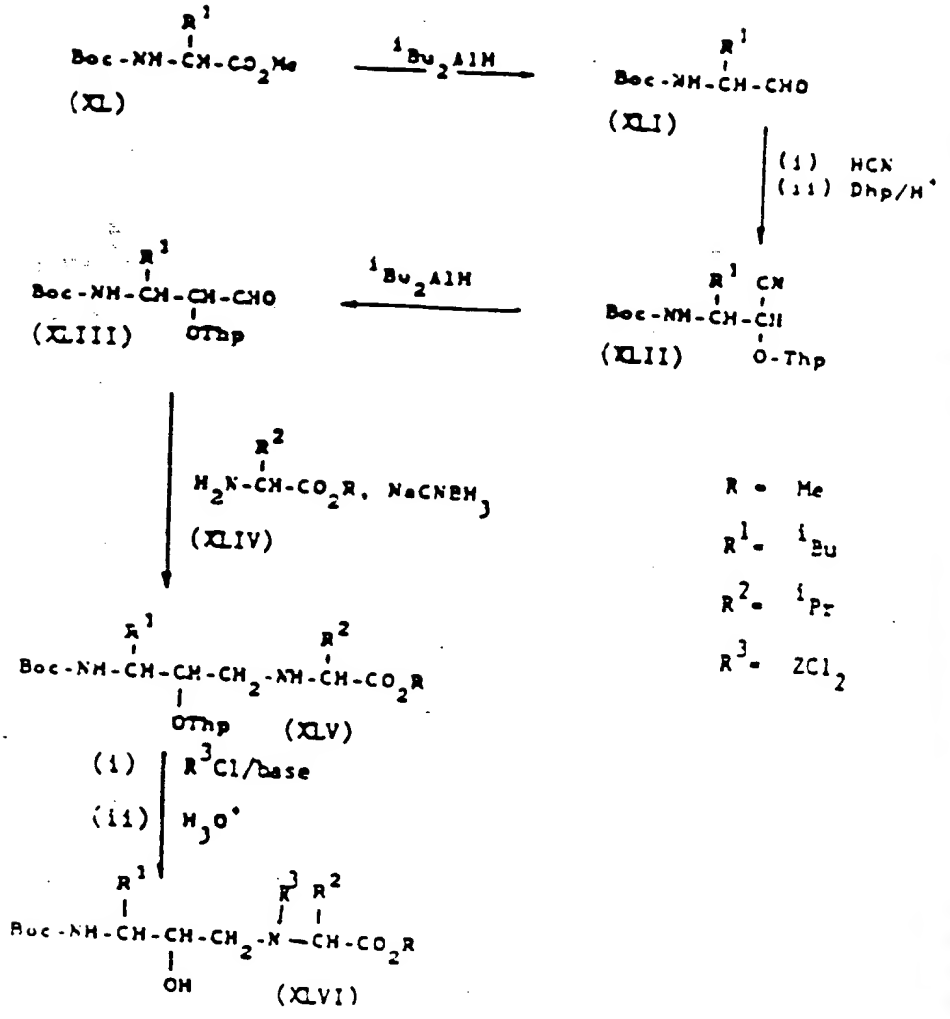
and coupling the latter to the tripeptide ester LVI.

8 By coupling $Boc\text{-}NH\text{-}CHR^1\text{-}CH_2\text{-}CO_2H$ obtained by one cycle of the Arndt-Eistert reaction from $Boc\text{-}NH\text{-}CHR^1\text{-}CO_2H$,) to the tripeptide ester LVI.

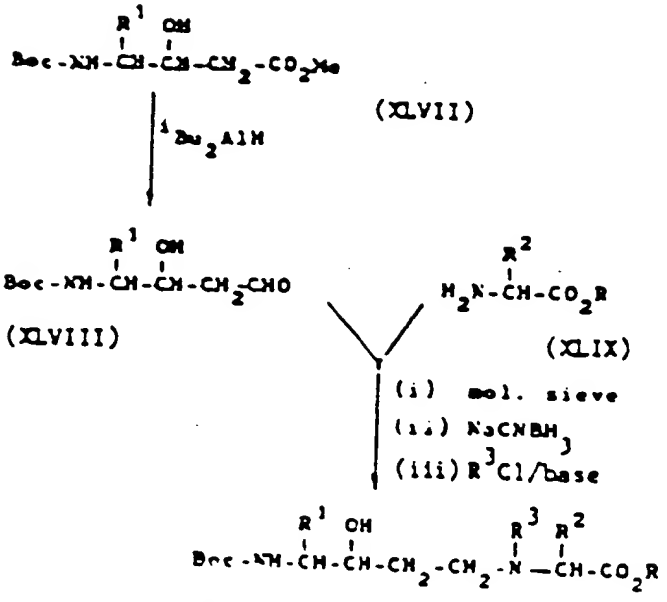
9 By oxidising the hydroxyl group in compound XLVI (Scheme I, R = H) to a keto group and coupling the resultant dipeptide analogue to the dipeptide ester LVII.

The same methods are applicable in making compounds corresponding to that of Example 2.

Scheme 1



Scheme 11



R = Me or Bzl

R^1 = i_{Bu}

R^2 = i_{Pr}

R^3 = $2Cl_2$

In the above syntheses 1 to 9 and Schemes I and II:

Boc = tert-butoxy carbonyl
DCCI = N, N^1-dicyclohexyl-carbodi-imide
HOBt = 1-hydroxybenzotriazole
Bom = π-benzyloxymethyl
Dhp = 2,3-dihydro-4H-pyranyl
Thp = 2-tetrahydropyranyl
ZCl_2 = 3,4-dichlorobenzyloxycarbonyl

EXAMPLES

The following are the fully detailed examples referred to earlier. The activity of H-176 (Example I) has already been given. Preliminary results for H-189 (Example II) indicate still higher activity, IC_{50} 0.009 μM against human renin and 0.012 μM against baboon renin.

Example 1

H-176 H-His-Pro-Phe-His-Sta-Val-Ile-His-OH (XXII)

Boc-His (π-Bom)-O-Resin (1g, 0.2 mmol) was washed with reagents as described in European Application 0 045 565 Example I. Subsequent deprotections and coupling reactions were carried out by using the same sequence of washes and reaction times with the following modifications.

During the coupling of Boc-Sta-OH to H-Val-Ile-His-(π-Bom)-O- Resin, the reaction was allowed to proceed for 16 hrs and 0.27 mmoles of Boc-statine were used. For all the other coupling reactions, 0.8 mmoles of Boc-amino acids were used.

After the final coupling, followed by acetylation for 1 hr, the resin was washed and dried to give 0.926 g of product.

This material was treated with HF at 0° for 1½ hrs. in the presence of anisole (1 ml) and dried overnight over potassium hydroxide. The resin was washed with acetic acid/water (1:1) (100 ml) to remove the peptide. Evaporation of volatiles afforded a residue which upon drying over KOH under high vacuum weighed 126 mgs.

This material was applied to a Sephadex G-25 column (82 x 2.5 cm), eluted with 10% acetic acid at 19.2 ml/hr, collecting 6.4 ml fractions. Fractions 27-39 were pooled, evaporated and the residue dried over KOH under high vacuum (67.5mg). This residue was applied to a CM-52 ion-exchange column (30 x 1 cm) and eluted with an ammonium acetate

gradient 0.05M to 0.5M over 2 days at 5.8 ml/hr collecting 2.9 ml fractions. After collecting 50 fractions, no peak assignable to the product was obtained on the trace. Therefore, the column was eluted with 0.5M ammonium acetate taking 5.8 ml fractions. The product from fraction 55 was found to be pure. Lyophilisation afforded 7.4 mgs of pure material.

H-176 $C_{60}H_{82}O_{10}N_{14}$ MW: 1099.356

TLC: Rf = 0.4 in EtoAc/Py/AcoH/H_2O (20:20:6:11) on silica plates

Amino acid analysis: after hydrolysis in 6N HCl plus phenol for 92 hrs, 110°, peptide content 88.6%

His: 2.97; Pro: 1.1, Val: 0.98; Ile: 0.97; Phe: 0.99

Example 2

H-189, H-Pro-His-Pro-Phe-His-Sta-Val-Ile-His-Lys-OH (XXXVI)

Solid-phase synthesis of this peptide was carried out by the described procedure starting with 1.2 grams (0.24 mmole) of Boc-Lys (Cl_2Z)-O-Resin. In the subsequent coupling reactions, 1 mmole of Boc-amino acid was used except for Boc-Statine of which 0.36 mmoles were used. The dried peptide-Resin ester weighed 1.53 grams.

0.6 grams of this material were subjected to HF + anisol treatment and afforded a residue of 171.6 mgs. This residue was applied to a G-25 Sephadex column (82 x 2.5cm) eluted

with 50% acetic acid at 19.2 ml/hr collecting 6.4 ml fractions. Fractions 32 - 42 provided 121.6 mgs of residue. Half of this material was applied to a CM-52 ion-exchange column (30 x 1cm), eluted with an ammonium acetate buffer gradient from 0.05M to 1M over 2 days at 5.8 ml/hr collecting 2.9 ml fractions. Fraction 33 afforded pure product which upon lyophilisation gave 9.5 mgs of material.

H-189 $C_{66}H_{101}O_{12}N_{17}$ MW: 1204.535

TLC (silica) Rf = 0.5 in EtoAc/Py /AcoH/H_2O (15:20:6:11)

Amino acid analysis after hydrolysis with 6N HCl + phenol at 110°/40 hrs:

His : 3.03; Ile : 0.90; Phe : 0.86; Pro : 2.19; Val : 0.94

Peptide content 80.1%.

Use of the compounds

Either of the above compounds may be given, as described earlier herein, in amounts of for example 0.1 mg/kg body weight daily in diagnosis of high-renin states and in therapy of heart failure and hypertension.

CLAIMS:

1. Polypeptide analogues of the general formula:

X-Y-Pro-Phe-His-A-B-Z-W (V)

or the partial sequences

X-A-B-Z-W (V) (i)

X-His-A-B-Z-W (V) (ii)

X-Phe-His-A-B-Z-W (V) (iii)

X-Pro-Phe-His-A-B-Z-W (V) (iv)

where

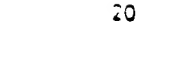
X = hydrogen, an amino-nitrogen protecting group or an amino acyl residue;

Y = a D- or L- basic or aromatic amino-acyl residue;

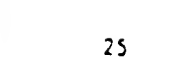
A =  (VI) where

G = (VII) with n = 0, 1, 2, 3

 (VIII) n = 1, 2, 3, 4

 (IX) n = 0, 1, 2, 3

 (X) n = 1, 2, 3, 4

 (XI) n = 1, 2, 3

 (XII) n = 3, 4

and where the configuration at asymmetric centres * is either R or S and R^1 and R^2, the same or different, are amino acid side chains;

R^3 = hydrogen or an amino-nitrogen protecting group;

R^6 = hydrogen or a hydroxyl protecting group;

B = a lipophilic amino acyl residue;

Z = an aromatic amino acyl residue;

and W = a hydroxyl group, an amide nitrogen group, or a basic amino acyl residue or amino alcohol residue derived therefrom;

or Z + W = the amino alcohol residue corresponding to the amino acyl residue Z.

2. A polypeptide analogue according to claim 1, wherein one or more of said Pro Phe and His are substituted in the ring, Pro by OH, Phe by OH, F, Cl, Br or Me, His by Me, or are replaced by, respectively, 4-hydroxyproline (HPro) or pGlu; Tyr, Phe(4-Cl) or Phe(4-F); His (Me) or spinacin.

3. A polypeptide analogue according to claim 1 or 2 wherein said protecting group X is an acyl or lower alkyl group and particularly said acyl group is acetyl, pivalcyl, t-butoxycarbonyl (Boc) benzyloxycarbonyl or benzoyl and said lower alkyl group is a C_1-C_5 alkyl group.

4. A polypeptide analogue according to claim 1 or 2, wherein said amino acyl group X is D- or L- Pro, Val or Ile, or Gly, and/or is in protected form carrying a group as set out in claim 3.

5. A polypeptide analogue according to any preceding claim, wherein said amino acyl residue Y is D- or L-His.

6. A polypeptide analogue according to any preceding claim, wherein said amino acid side chains R^1 and R^2 are lipophilic or aromatic, e.g. isopropyl, isobutyl and benzyl, and particularly those of Leu Ile Val Phe cyclohexyl-Ala adamantyl-Ala, or Phe Tyr Trp or His.

7. A polypeptide analogue according to any preceding claim, wherein said protecting group R^3 is lower alkyl, lower acyl, $-SO_2Ph$, $-SO_2C_6H_4CH_3(p)$, t-butoxy carbonyl (Boc), benzyloxycarbonyl or ring substituted benxyloxycarbonyl, and particularly wherein said alkyl or acyl group is a C_1-C_5 group.

8. A polypeptide analogue according to any preceding claim, wherein said hydroxyl protecting group R^6 is lower alkyl or lower acyl, particularly C_1-C_5 alkyl or acyl ,or benzyl or tetrahydropyranyl.

9. A polypeptide analogue according to any preceding claim, wherein said residue B is a D- or L-Val Leu or Ile residue.

10. A polypeptide analogue according to any preceding claim, wherein said residue Z is a D- or L-Tyr Phe or His residue.

11. A polypeptide analogue according to any preceding claim, wherein said hydroxyl group W is protected in ester form, e.g. by a lower alkyl or cycloalkyl group, or Bzl, and particularly wherein said lower alkyl group is a C_1-C_5 alkyl group and said cycloalkyl group a C_3-C_7 cycloalkyl group.

12. A polypeptide analogue according to any preceding claim, wherein said amide nitrogen group W is in protected form, especially i) a group $-NHR^5$ or $-N(R^5)_2$ where R^5 is lower alkyl and $(R^5)_2$ is two lower alkyl groups the same or different or a cycloalkyl group, particularly where the or each said alkyl group is a C_1-C_5 alkyl group and said cycloalkyl group is a C_3-C_7 cycloalkyl group

or ii) a group $-NH-(CH_2)_n-Q$ or $NR^5-(CH_2)_n-Q$ where n = 2 to 6 and Q = an amino or guanidyl group where R^5 is lower alkyl, particularly where one or more of the hydrogens attached to nitrogen in said protecting group is replaced by R^5 or $(R^5)_2$, defined as above.

13. A polypeptide analogue according to any preceding claim, wherein said amino acid residue W is a D- or L- Ser, Ile or Arg residue as such or as

i) an amide or protected amide particularly when protected by a group as set out in claim 12, or

ii) an ester particularly when the esterifying group is as set out in claim 11.

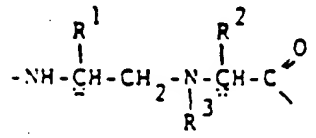
14. A polypeptide analogue according to any preceding claim, wherein said amino alcohol residue W

is the amino alcohol residue derived from D- or L- Ser, Lys or Arg or said amino alcohol residue Z + W is derived from D- or L- Tyr Phe or His, in either case as such or protected in ester or ether form, e.g. by a protective group R^6 as set out in claim 8.

15. A polypeptide analogue according to any preceding claim, wherein G is selected from:

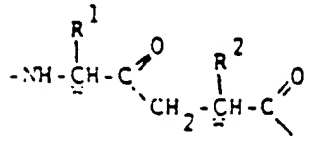
1		(XXVI)
2		(XXVII)
3		(XXVIII)
4		(XXIX)
5		(XXX)
6		(XXXI)
7		(XXXII)
8		(XXXIII)
9		(XXXIV)

16. A polypeptide analogue according to any preceding claim, wherein said polypeptide analogue is in further modified form by isosteric replacement of one or more remaining peptide bonds by



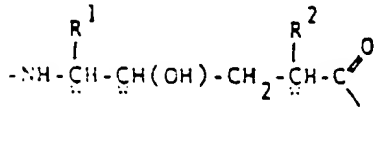
(XIII) "reduced" isostere bond

or



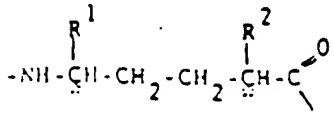
(XIV) "keto" isostere bond

or



(XV) "hydroxy" isostere bond

or



(XVI) "hydrocarbon" isostere bond

where the significance of *, R^1, R^2 and R^3 is as before, particularly where one or both of the Pro-Phe and Phe-His bonds in formula V is the site of the isosteric replacement.

17. A polypeptide analogue according to any preceding claim, wherein said polypeptide analogue is in protected form at one or more remaining amino or amide (including peptide) nitrogen, carboxyl, hydroxy or other reactive groups, or in salt form at amino, imidazole or carboxyl groups, or is as an acid addition salt at one or more basic centres.

18. Polypeptide analogue of the general formula:

$$X\text{-}Y\text{-}Pro\text{-}Phe\text{-}His\text{—}A\text{—}B\text{—}Z\text{—}W \qquad (V)$$
$$6\ \ 7\ \ 8\ \ 9\ \ 10,11\ \ 12\ \ 13$$

or the partial sequences:

X-A-B-Z-W (V) (i)

X-His-A-B-Z-W (V) (ii)

X-Phe-His-A-B-Z-W (V) (iii)

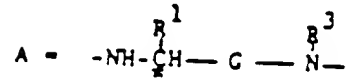
X-Pro-Phe-His-A-B-Z-W (V) (iv)

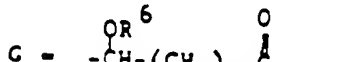
where:

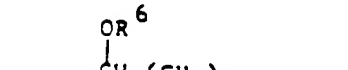
Pro, Phe, and His may be in substituted form, e.g. carrying OH, F, Cl, Br or Me;

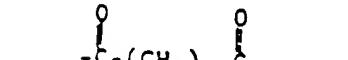
X = H, or an acyl or other N-protecting group e.g. acetyl, pivaloyl, benzyloxycarbonyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1-C_5); or an D- or L- amino acyl residue (especially Pro), which may itself be N-protected similarly:

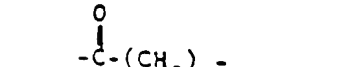
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;

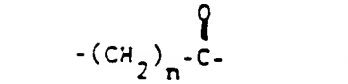
A =  (VI) where

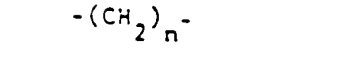
G =  (VII) with n = 0, 1, 2, 3

 (VIII) n = 1, 2, 3, 4

 (IX) n = 0, 1, 2, 3

 (X) n = 1, 2, 3, 4

 (XI) n = 1, 2, 3

 (XII) n = 3, 4

and where the configuration at asymmetric centres * is either R or S, R^1 and R^2, the same or different, being = ^{i}Pr (isopropyl), ^{i}Bu (isobutyl), Bzl (benzyl) or other amino-acid side chain preferably lipophilic or aromatic;

R^3 = H; lower alkyl (C_1-C_5); or t-butyloxycarbonyl, benzyloxycarbonyl, ring substituted benzyloxycarbonyl, $-SO_2PH$, $-SO_2C_6H_4CH_3(p)$, formyl or other N-protecting group including lower acyl (C_1-C_5) generally;

R^6 = H, or lower alkyl, lower acyl, benzyl, tetrahydropyranyl, or other hydroxyl protecting group;

B = D- or L- Val, Leu or Ile or other D- or L- lipophilic amino-acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D- aromatic amino-acyl residue;

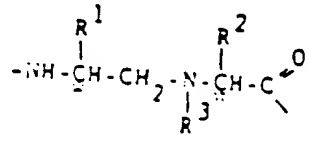
and W = i)-OH as such or in protected ester form e.g. as $-OR^4$ where R^4 = lower alkyl primarily C_1-C_5 and particularly ^tBu, or cycloalkyl primarily C_3-C_7, or Bzl, or other ester forming group; or ii)$-NH_2$, as such or in protected amide form as $-NHR^5$ or $-N(R^5)_2$ (where R^5 = an N-protecting or other substituent group e.g. lower alkyl as for R^4 and $(R^5)_2$ = two such groups or e.g. cyclo-alkyl, primarily C_3-C_7), or as $-NH-(CH_2)_n-Q$ or $-NR^5-(CH_2)_n-Q$ (where n = 2 to 6 and Q = NH_2 or $-NH-C(=NH)-NH_2$ and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R^5)_2$); or iii) an D- or L- serine or D- or L- lysine, arginine or other basic amino-acyl residue as such or in amide form, substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case

may be; or iv) an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above

or

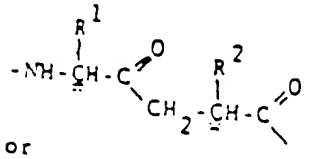
Z + W = an alcohol derived from L- or D- Tyr, Phe or His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds, for example by reduced $-CH_2-NH-$, keto, $-C(=O)-CH_2-$, hydroxy, $-CH(OH)-CH_2-$, or hydrocarbon $-CH_2-CH_2-$ isosteric links in the form:



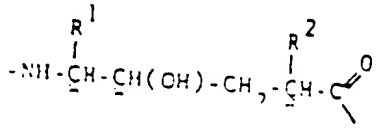
(XIII) "reduced" isostere bond

or



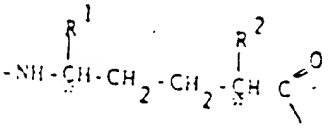
(XIV) "keto" isostere bond

or



(XV) "hydroxy" isostere bond

or



(XVI) "hydrocarbon" isostere bond

where the significance of *, R^1, R^2 and R^3 is as before;

and said polypeptide further being in free form or in protected form at one or more remaining amino or amide (including peptide) nitrogen, carboxyl, hydroxy or other reactive groups, or in salt form at amino, imidazole or carboxyl groups, in particular as their physiologically acceptable acid addition salts at basic centres.

19. The polypeptide analogues:

H-His-Pro-Phe-His-Sta-Val-Ile-His-OH

H-Pro-His-Pro-Phe-His-Sta-Val-Ile-His-Lys-OH

or corresponding analogues wherein the $-CH(OH)CH_2CO-$ link of the statine residue is replaced by one of the links set out in claim 15.

20. When for use in a diagnostic test for high renin states wherein a polypeptide analogue is administered, a positive result being indicated by a fall in blood pressure related to said administration, or for use in treatment of heart failure or hypertension wherein an effective amount of a polypeptide analogue is administered, a polypeptide analogue according to any preceding claim.

21. A pharmaceutical composition comprising a polypeptide analogue according to any preceding claim 1 to 19, in a pharmaceutically acceptable medium.

European Patent Office

EUROPEAN SEARCH REPORT

EP 83305353.1

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.)
P,X	EP - A2 - 0 077 028 (MERCK & CO. INC.) (20-04-1983) • Pages 14,15, especially lines 24,32; claims 1,6 • --	1,3-5, 9,10, 15,17, 18,21	C 07 C 103/52 C 12 Q 1/36 A 61 K 37/02
D,Y	EP - A1 0 045 665 (HALLETT) • Claims 1,30-33 • --	1,3,5, 9,10, 17,18, 20,21	
P,Y	EP - A1 - 0 081 783 (MERCK & CO. INC.) (22-06-1983) • Claims 1,3,5; pages 13-16 • ----	1,3,5, 9,10, 17,18, 21	

TECHNICAL FIELDS SEARCHED (Int. Cl.)
C 07 C 103/00
C 12 Q

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	30-11-1983	PETRUSSEK

CATEGORY OF CITED DOCUMENTS

X particularly relevant if taken alone
Y particularly relevant if combined with another document of the same category
A technological background
O non-written disclosure
P intermediate document

T theory or principle underlying the invention
E earlier patent document, but published on, or after the filing date
D document cited in the application
L document cited for other reasons

& member of the same patent family, corresponding document